A COMPARATIVE INVESTIGATION OF THE SHRINKAGE OF UNFIXED AND PREPARED NUCLEI OF LIVER AND KIDNEY TISSUE OF WHITE MICE

A Thesis

Presented to

The Graduate Division

Drake University

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts in Biology

---

by

Edwin L. Brunsting

August 1964

A COMPARATIVE INVESTIGATION OF THE SHRINKAGE OF UNFIXED AND PREPARED NUCLEI OF LIVER AND KIDNEY TISSUE OF WHITE MICE

by

Edwin L. Brunsting

Approved by Committee:

Rodney G. Rogers
Chairman

Leland P. Johnson

F. Emmett Jacob

Dean of the Graduate Division

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Whenever cells are studied, a microscopist endeavors to study them in as near their natural condition as possible. While the study of living or fresh material is desirable, it can be carried on only to a limited extent. Most structures of the animal body, though opaque, must be examined largely by transmitted light, hence, special preparation is necessary to put them into suitable condition. This is accomplished by cutting them into thin slices (section method).

In most instances, the minute structure of a tissue of an organism can be studied to the best advantage only after the application of certain agents which serve to emphasize the various structural elements. A tissue so prepared is an artificial product, in that it is not exactly the same as it is in the living organism. Recent studies of protoplasm in unfixed conditions have strengthened the belief that many reagents preserve very faithfully the actual structure of the cell content.

A quantitative comparison of the nuclear diameters and volumes of living and fixed nuclei has not been made. This study was undertaken to further the knowledge of nuclear diameters and volumes in procedures that alter the

nuclear structure and appearance.

The purpose of this study was to determine the relative diameters and volumes of unfixed and fixed nuclei of hepatic and renal cells of Swiss Strain white mice.

CHAPTER II

HISTORY

Much work has been done on biological stain research of nuclei in such fields as microtechnique, microscopy, bacteriology, and cytology. In the literature, nuclear sizes were usually presented for isolated, fixed nuclei, but a size for a living nucleus was seldom found.

The phenomenon of shrinkage and swelling of nuclei has been a question in histochemistry for many years. The unanswered question involved an argument of chemical and physical forces which caused a stain to react with a nucleus. The chemical theory was dependent largely upon the acidic or basic character of the dye molecule. Certain parts of animal and plant cells are acid in character and, hence, have an affinity for the basic dyes. The nuclei of the cells, especially the chromatin within the nuclei, have an acidic nature due to their constituent nucleic acids. The dye and nucleic acids would combine in a chemical reaction.[1]

The physical theory of staining implies three factors: (1) cells are porous and simple physical forces such

---

[1]H. J. Conn, Biological Stains (Geneva, New York: Biotech Publications, 1946), p. 208.

as capillarity and osmosis could account for penetration of the dye; (2) the action of adsorption could account for the many staining phenomena; and (3) a dye might penetrate some cellular elements by absorption, remaining there in a state of solid solution. Any one or all of these factors might operate at any given instance.[1]

Neither theory could explain the staining phenomena. A dye penetrated different cells equally readily, but could be extracted from some while scarcely at all from others. It was assumed that dyes penetrated by mere absorption and diffusion, but were precipitated there by acids or bases. Such a theory admitted the possibility of chemical action. In a chemical union, a new substance was formed which did not have the properties of either substance entering into its formation. When a tissue was stained, there was no evidence of any new substance having been formed; the colored tissue merely took on the characteristic of the dye (color).[2]

Stearn and Stearn noted that basic dyes caused an increase in mass when added to a solution containing nucleic acid. Acid dyes had no effect on nucleic acid solutions. They pointed out that this was due to the dominance of chemical forces in colloidal adsorption. They treated basic

---

[1] Ibid., p. 210.

[2] Ibid.

dye solutions with typical proteins and noted an increase in mass; acid dye solutions behaved in an opposite direction. They postulated that there was a definite reaction between dye ions and the oppositely charged ions of protein and nucleic acids.[1]

Cowdry also noted the coagulating action of the fixative upon materials in the nucleus. He noted that a morphological change occurred in sections after fixation which showed a heterogenicity, or diversity, in examination of the nuclei of living cells.[2]

Tolstoouhov investigated several fixing fluids and postulated that many fixing fluids gave stable compounds with the tissue proteins which almost permanently change the chemical composition. He noted that formalin forms inert compounds with amino acids, thus making the tissue more acid (i.e., it lowers the pH value).[3]

Tolstoouhov studied the staining properties of

---

[1]A. E. Stearn and E. W. Stearn, "The Mechanism of Staining Explained on a Chemical Basis. I. The reactions between dyes, proteins, and nucleic acids," Stain Technology, IV (October, 1929), 111.

[2]E. V. Cowdry, Microscopic Techniques in Biology and Medicine (Baltimore: The Williams and Wilkins Company, 1943), p. 140.

[3]A. V. Tolstoouhov, "The Effect of Preliminary Treatment (Fixing Fluids) on Staining Properties of Tissues," Stain Technology, III (April, 1928), 49.

different organs of the same animal fixed in the same fluid and was able to find differences in the chemical composition of the different stains which will be taken up.[1] Tolstoouhov believed that the staining properties of tissues were dependent upon chemical composition. Staining properties depend on: (1) strength of the dye, (2) strength of the tissue proteins or isoelectric points, and (3) the pH-value of the dye solution.[2]

The preparation of animal tissues for microscopic study presents a difficult problem to the histologist and pathologist. Tissues must be treated in such a way that their structures can readily be visualized under the microscope. It is important that they be altered as little as possible during treatment if one is to obtain a view of their basic structure comparable to that of the living state.[3]

According to Birge and Tibbitts, pronounced shrinkage not only resulted in substantial distortion of cell and tissue structure but also reduced the dimensions of fine detail below the limits resolvable by the light microscope.[4]

---

[1] Ibid.

[2] Ibid., p. 50.

[3] H. R. Koenig, A. Groat, and W. F. Windle, "A Physiological Approach to Perfusion Fixation of Tissues with Formalin," Stain Technology, XX (January, 1945), 13.

[4] Wesley J. Birge and P. Donald Tibbitts, "The Use of

The passage of a tissue section through a series of microtechnique methods can have serious effects in the form of swelling and shrinkage. Each step in this series can distort the cell components of both cytoplasm and nucleus.

McClung considered the process of fixation as one of the most important in the series; upon its character all the remaining steps and ultimate result depend. His endeavor here was to preserve in a permanent form, as nearly as possible, the exact configuration of cells and tissues in the living state. He found that this cannot be exactly accomplished because living protoplasm is a gelatinous or semi-solid fluid material and after fixation it becomes a solid. He found this to be accomplished by the process of coagulation.[1]

According to Koenig, Groat, and Windle, the double duty of preserving and preparing tissue for processes to follow rested upon fixation. They stated that fixation involved the denaturation of tissue proteins and this prevented the post mortem changes of autolysis and bacterial decomposition.[2]

Sodium Chloride-Containing Fixatives in Minimizing Cellular Distortion in Histological and Cytochemical Preparations," Journal of Histochemistry and Cytochemistry, IX (1961), 409.

[1]C. E. McClung, Handbook of Microscopical Technique (New York: Paul B. Hoeber, Inc., 1937), p. 8.

[2]Koenig, Groat, and Windle, loc. cit.

Birge and Tibbitts considered that one of the principal objections to the fixation of tissues by chemical means was the inadequate stabilization of the cells against shrinkage at the time of fixation. Hertwig, as cited by Birge and Tibbitts, demonstrated that the normal volume of amphibian cartilage cell nuclei was decreased by more than 50% following formalin fixation, ethyl alcohol dehydration, and paraffin embedding.[1]

Ross found that the fixative is probably responsible for most of the shrinkage in a preparation.[2] Cowdry stated the advantages and disadvantages of fixation. He considered that the normal form relations of the tissue components were preserved more faithfully in large pieces because it was not necessary to separate the tissue by teasing to obtain thin or small pieces for microscopic study; the cells were suddenly and uniformly killed. His chief objection to fixation was the modification of structure; thus, care must be exercised in reaching conclusions concerning living tissues from the study of fixed ones.[3]

---

[1]Birge and Tibbitts, loc. cit.

[2]K. F. A. Ross, "Cell Shrinkage Caused by Fixatives and Paraffin-Wax Embedding in Ordinary Cytological Preparations," Quarterly Journal of Microscopical Science, XCIV (1953), 125.

[3]Cowdry, op. cit., p. 74.

The reagents chosen for fixation should be adapted to the purpose for which the material is to be used. Some fixatives preserve nuclear conditions, others those of the cytoplasm. The particular fixative that may best be used depends upon the nature of the material. In some cases, this requires a reagent which penetrates with extreme vigor and rapidity. McClung indicated that one of the picro-formol-acetic acid mixtures may be selected for easy penetration of a visceral organ. Bouin's picro-formol is an example of this type.[1]

The three substances, picric acid, acetic acid, and formalin, have individually different effects upon protoplasm. Gray indicated that the preservation of nuclear detail was related to that of penetration. In working with the most universally employed penetrating agent, acetic acid, he noted that this organic acid caused a swelling of the tissue.[2] Birge and Tibbitts found that acetic acid seemingly was not effective in precipitating and stabilizing most tissue proteins. They found it had a swelling effect on proteins and biocolloidal systems. They considered this swelling was brought about primarily by the imbibition of

---

[1]McClung, op. cit., p. 9.

[2]Peter Gray, Handbook of Basic Microtechnique (New York: McGraw-Hill Book Company, Inc., 1958), p. 83.

additional water attracted to the protein molecules by charged hydrophilic groups set free as a result of the acid treatment.[1] Even though acetic acid did not shrink the tissue, Ross observed that it permitted shrinkage of tissues by subsequent processes.[2] Tarkhan found that tissues fixed in acetic acid or in mixtures containing it swell on being put into water. He observed that fixatives which contain acetic acid were dehydrated, whereas lower grade alcohols are liable to cause the tissues to swell.[3] McClung noted that formalin had a swelling action.[4] Patten and Philpott, working with pig embryos, found a 4.8% increase in the length of the embryos when fixed in formalin.[5] Lassek and Powers experimented with concentrated and 10% formalin and postulated that the concentrated fixative was better because it had a greater speed of penetration without causing additional distortion or artefacts.[6] Tarkhan noted that

---

[1]Birge and Tibbitts, op. cit., p. 413.

[2]Ross, op. cit., p. 136.

[3]A. A. Tarkhan, "The Effect of Fixatives and Other Agents on Cell Size and Tissue Bulk," Journal of Royal Microscopical Society, LI (1931), 392.

[4]McClung, loc. cit.

[5]B. M. Patten and R. Philpott, "The Shrinkage of Embryos in the Processes Preparatory to Sectioning," Anatomical Record, XX (March, 1922), 407.

[6]A. M. Lassek and M. M. Powers, "Concentrated Forma-

formol, when used in mixtures, augmented the penetration of those fixatives which had a shrinking power.[1] Koenig, Groat, and Windle found that tissues fixed in 10% formalin gained in weight in a range from 1% to 6.2%. They reasoned that this increase in mass came from the filtration of the fluids into the tissues.[2]

Koenig, Groat, and Windle experimented with hypotonic and hypertonic fixing fluids. They found that if the fixing fluid is not isotonic, hypotonicity of the fixing fluid to the intracellular fluid would produce hydration and swelling of the cells and hypertonicity to the intracellular fluid would produce dehydration and shrinkage. In addition to the separation of the tissue elements consequent to an increase in interstitial fluid, they found a distortion of the cellular elements. If the fixative was hypertonic, they observed that the distortion of the tissue was that of shrinkage.[3]

A 10% formalin fixative is a hypotonic solution. Koenig, Groat, and Windle considered that this solution

---

lin Versus a 10% Solution as a Fixative Preceding Silver Staining," Stain Technology, XXIV (January, 1949), 34.

[1]Tarkhan, loc. cit.

[2]Koenig, Groat, and Windle, op. cit., p. 15.

[3]Ibid.

distributed itself so that its concentration in the intracellular fluid and interstitial fluid would be equal. Water then diluted the interstitial fluid and produced a swelling of the cells.[1]

Picric acid in concentrated aqueous solution shrinks tissue, such as liver, during fixation. Guyer explained this shrinkage by considering that picric acid acted by coagulating or precipitating various constituents of tissues.[2] Ross tested several fixatives; he considered picric acid and ethyl alcohol to have the greatest shrinking effect of simple fixatives.[3]

Birge and Tibbitts found that Bouin's solution produced a greater effectiveness than formalin in stabilizing the cellular cytoplasm. They reasoned that acetic acid diffused into tissue blocks ahead of active fixing agents.[4]

Birge and Tibbitts added small quantities of NaCl to fixatives to minimize tissue distortion and shrinkage. They found a mean volume of 127 cubic microns and 227 cubic microns for 2n and 4n nuclei of the liver when fixed in

---

[1] Ibid.

[2] Michael F. Guyer, Animal Micrology (Chicago: The University of Chicago Press, 1953), p. 15.

[3] Ross, op. cit., p. 135.

[4] Birge and Tibbitts, loc. cit.

Bouin's solution and 140 cubic microns and 243 cubic microns for tissues fixed in Bouin/NaCl solution. Therefore, the tissue shrinkage was reduced 7%.[1] Birge and Tibbitts found that Bouin's solution had 13% less cytoplasmic shrinkage than formalin fixed material.[2] Baker, as cited by Birge and Tibbitts, considered that the over-all tonicity of the fixative was not as important in tissue shrinkage as the concentration of small ions. He postulated that the solvent phase of the fixative may penetrate the tissue more rapidly than large ions and this would expose the tissue to a hypotonic solution just prior to fixation.[3] According to Young, as cited by Birge and Tibbitts, the swelling incurred may result in the bursting of the cell if small ions of NaCl were added, since these would diffuse more rapidly and minimize the imbalance in tonicity between extra- and intracellular environments.[4]

Ross, working with cells and nuclei of the primary spermatocytes of the snail Helix aspera, made a series of measurements after applying various fixatives.[5] He found that the per cent shrinkage in nuclear diameter and volume for Bouin's, Zenker's, chromic acid, and neutral formalin was 23±7% and 54±12% respectively. Picric acid, ethyl

---

[1] Ibid. [2] Ibid. [3] Ibid., p. 412. [4] Ibid.

[5] Ross, op. cit., p. 125.

alcohol, and trichloroacetic acid produced 34±6% nuclear shrinkage in diameter and 71±8% shrinkage in nuclear volume.[1]

Patten and Philpott measured pig embryos after fixation in Bouin's fixative. They determined the shrinkage to be very slight (average 2.5%) and in the remainder of the process shrinkage was gradual and not excessive. They noted that the preservation of cytological detail by Bouin's fixative is greatly superior to that secured by formalin or formol-alcohol.[2]

Tarkhan found that tissues possessed a more or less limited power of shrinkage, which, if exhausted in the fixative, cannot manifest itself to the same extent during dehydration and other technique procedures.[3] Patten and Philpott noted that the greater the shrinkage by fixation, the less the subsequent shrinkage during hydration, and, conversely, a relative lack of shrinkage during fixation was followed by an increased loss of volume during subsequent dehydration processes. They considered the supposed advantages of a mixture, such as Bouin's, would be nullified by the subsequent shrinkage.[4]

---

[1] Ibid., p. 137.

[2] Patten and Philpott, op. cit., p. 412.

[3] Tarkhan, loc. cit. [4] Patten and Philpott, loc. cit.

Washing is the process of removing excess fixative from the specimen. Following the use of an aqueous fixative, water is used in most cases to do the washing. McClung noted that when picric acid was used in the fixative, washing must take place in 70% alcohol in order to avoid maceration. He asserted that any picric acid which remained in the specimen would interfere with staining. In most instances, washing did not distort the tissue.[1]

The shrinkage of tissues continues during dehydration. Tarkhan noted that absolute alcohol was perhaps the most powerful simple fixative and a brutal dehydrating agent. He considered the hardening of tissues due to the coagulation of their proteins and withdrawal of water. He found most of the shrinkage took place in the first 2-3 hours and gradual decrease followed. He found that dehydration following the use of Flemming's fixative resulted in a total shrinkage in diameter of a nucleus of 9%. A one-tenth reduction in diameter resulted in a very great reduction in volume.[2]

Tellyesniczsy, as cited by Tarkhan, asserted that greater tissue changes occurred on transfer of tissue from the fixative to other media than during fixation itself.[3]

---

[1]McClung, op. cit., p. 11.

[2]Tarkhan, op. cit., p. 392.

[3]Ibid.

Tarkhan, using Susa's fixative, which contains acetic acid, found swelling when tissue was transferred to 50% alcohol and this continues in the 70% and 90% alcohols. He found shrinkage in the tissue when placed in 96% alcohol and absolute alcohol which compensated for the swelling in the lower grades of alcohol.[1] Following formol fixation, Tarkhan observed no shrinkage in 70% alcohol; thus, preservation is justified in this grade of alcohol.[2] Alcohol and water, because of the spaces among the atoms of the alcohol molecule in which the molecules of water arrange themselves, contract upon mixing to further increase tissue shrinkage. Patten and Philpott noted that pig embryos that passed from 10% formalin to 70% alcohol had a shrinkage of 5.2%, to 95% alcohol had a shrinkage of 3.8%; the second change of absolute alcohol caused an additional shrinkage of 2.1%.[3] Patten and Philpott observed a shrinkage of 1.5% when transferred from Bouin's to 70% alcohol and a 2% shrinkage when placed in 95% alcohol. They observed a shrinkage of 5% when transferred from 95% alcohol to absolute alcohol.[4]

One of the most universally used clearing agents is xylol. Tarkhan has demonstrated that considerable shrinkage

---

[1] Ibid., p. 391. [2] Ibid., p. 392.

[3] Patten and Philpott, op. cit., p. 406.

[4] Ibid., p. 410.

is seen to occur. He noted that xylol shows maximum shrinkage over toluol, benzol, and cedar-wood oil.[1] Patten and Philpott noticed a shrinkage of 0.5% when tissue was passed from absolute alcohol to xylol when 10% formalin was used as the fixative. They observed a 1% shrinkage when the pig embryos were passed from absolute alcohol to xylol following fixation in Bouin's picro-formol.[2]

Paraffin has the power of shrinking tissue. Tarkhan found that heat also induced shrinkage. He observed that paraffin contracted, and the longer the tissue remained in paraffin, the more the tissue contracted.[3] Ross noted that cells embedded in paraffin wax were more shrunken after being fixed with simple fixatives than when fixed with fixing mixtures, such as Bouin's.[4] Patten and Philpott used a 10% formalin fixative and observed a 4.2% shrinkage when the tissue was placed in paraffin. They used Bouin's fluid and observed a 6.5% shrinkage when placed in paraffin.[5] Ross noted a linear shrinkage of about one-third following paraffin embedding after the tissue was fixed by many of the

---

[1]Tarkhan, loc. cit.

[2]Patten and Philpott, loc. cit.

[3]Tarkhan, op. cit., p. 396.

[4]Ross, op. cit., p. 138.

[5]Patten and Philpott, loc. cit.

common fixing solutions. He postulated that the embedding process was one of the most important factors in determining final cell size.[1]

Over the entire range of microtechnique methods, a 30% reduction in tissue volume was evident. Stowell, working with rabbit kidney cortex, noted that Susa's and Bouin's fixatives gave the best general results in comparing various fixation, dehydration, and embedding techniques.[2] Stowell found in comparing fresh tissue of rabbit kidney with fixed, washed, dehydrated, and cleared tissue that Bouin's-xylol procedure produced a 14% shrinkage in volume with Bouin's fixative, 4% in 70% alcohol, 9% in xylol, and 8% when embedded; the final volume was 63% of the fresh tissue. He also noted when he used Petrunkewitche's-xylol procedure that the fixation caused a swelling of 17% from the fresh tissue, 13% increase in volume above that caused by the fixative when placed in 70% alcohol, a 51% shrinkage in volume when in the xylol, and a final shrinkage of 15% when embedded; the final volume was 64% of the fresh tissue. He used a 10% formalin-xylol procedure and observed that the 10% formalin caused a 34% increase in volume, the 70%

---

[1]Ross, loc. cit.

[2]Robert E. Stowell, "Effect on Tissue Volumes of Various Methods of Fixation, Dehydration, and Embedding," Stain Technology, XXVI (April, 1941), 67.

alcohol produced a 52% shrinkage, and xylol reduced the volume 18%; the final volume of the fixed tissue was 56% of the fresh tissue. Even though there was initial shrinkage and swelling in fixation, the percentage of shrinkage varied by 8% for the various procedures.[1]

Stowell cooled 500 cc. of parawax from 59° C. to 20° C. and found a 14.3% decrease in volume. He concluded that the paraffin, in shrinking more than the tissue, must exert a compressing force on it. He found that embedded tissue had not reached its maximum capacity to shrink and thus sectioning may produce further distortion.[2]

Aumonier found that thinner sections and wax of higher melting point gave greater shrinkage of tissues during cutting and fixing of the sections to the slide. He found that the shorter the time allowed for stretching the section resulted in no change.[3]

Aumonier noted that if paraffin sections did not show an increase in width to compensate for the increased length when the paraffin melted, these sections should have increased in thickness. He reasoned that paraffin sections, which are fastened relatively firmly to the slide by albu-

---

[1] Ibid., p. 74. [2] Ibid., p. 81.

[3] F. J. Aumonier, "Notes on the Distortion of Paraffin Sections," Journal of Royal Microscopical Society, LVIII (1938), 254.

min, would be free to change chiefly in thickness.[1]

Stowell rated fixatives and procedures and found that the Bouin-xylol procedure gave the best all-around results, best for nuclear fixation and hematoxylin staining, although cytoplasmic fixation was not good. He asserted that fixatives which produced the least volume change do not, however, give the best microscopical picture. He found that Zenker's fixative caused the greatest shrinkage but is widely used because of its excellent cytoplasmic preservation.[2]

Freeman, Moyer, and Lassek found that the pH of Bouin's fixative is very stable. They placed 1 gram of tissue in 25 cc. of fixative and 1 gram of tissue in 125 cc. of fixative and no change in pH occurred.[3]

Cowdry determined that the shrinkage of nuclei when examined in stained sections was generally more than 10% of their size *in vivo*.[4] Munzer, as cited by Wilson and Leduc, reported that sometimes the nuclei differ in staining reaction or density; one nucleus may be more basophilic and

---

[1] *Ibid*.

[2] Stowell, *op. cit.*, p. 79.

[3] B. L. Freeman, E. K. Moyer, and A. M. Lassek, "The pH of Fixing Fluids During Fixation of Tissues," *Anatomical Record*, CXXI (March, 1955), 599.

[4] Cowdry, *op. cit.*, p. 142.

the other more acidophilic.[1] Clara, as cited by Wilson and Leduc, said that the two nuclei may show a different density in Heidenhain's hematoxylin.[2] One may be so dense that the internal structure is not visible while the other is nearly destained. Wilson and Leduc believed this to be an artefact.

Wilson and Leduc stained mouse nuclei with Heidenhain's hematoxylin; some of the nuclei were all black and of small size in the 5-micron sections, but in sections which were considerably thicker than 5 microns there were both larger undifferentiated nuclei and a great number of the small black nuclei. The larger, less deeply stained nuclei were those which had been cut in sectioning.[3]

Zollinger stated that cells exposed to formalin, acids, alcohol, and acetone show a brilliant type of nucleus (i.e., their nuclei are irreversibly shrunken) and the nuclear membrane, the nucleoli, and the chromatin network are bluish in color when viewed under the phase microscope.[4]

---

[1] J. W. Wilson and E. H. Leduc, "Nuclear Phenomena in Mouse Liver," American Journal of Anatomy, LXXXII (May, 1948), 356.

[2] Ibid.

[3] Ibid.

[4] H. U. Zollinger, "Cytological Studies with the Phase Microscope. Alterations in the Nuclei of 'Resting' and Dividing Cells Induced by Means of Fixatives," American Journal of Pathology, XXIV (October, 1948), 801.

Cowdry stated that acidophilic material in cells may appear to be increased, for it is more concentrated owing to a decrease in volume.[1]

---

[1]Cowdry, loc. cit.

CHAPTER III

MATERIALS AND METHODS

The purpose of this study was to determine the relative diameters and volumes of unfixed and fixed nuclei of hepatic and renal cells of adult Swiss Strain mice obtained from Schettle Biologicals, Stillwater, Minnesota. Other fixatives and stains could be used, but only Bouin's picro-formol was utilized in the fixation of tissues and only hematoxylin was selected as the nuclear stain in order to reduce the scope of the problem.

Two adult white mice were used in the investigation. The mice were killed by a blow on the head. The entire liver was excised and a portion, approximately 5 mm. thick, 6 mm. broad, and 10 mm. long, was placed in a 100 ml. Erlenmeyer flask which contained Anderson's solution (0.0094M KH_2PO_4, 0.0125M K_2HPO_4, 0.0015M $NaHCO_3$, 0.145M Sucrose, in 1 liter of distilled water).[1] All of the above mentioned analytical reagents were purchased from Mallinckrodt Chemical Works, St. Louis. The chemicals were weighed on an analytical balance (#100, Voland and Sons, New Rochelle, New

---

[1] N. G. Anderson and K. M. Wilbur, "Studies on Isolated Cell Components. IV. The effect of various solutions on the isolated rat liver nucleus," Journal of General Physiology, XXXV (May, 1952), 791.

York).

A section razor (Clay-Adams, Inc., New York) was used to obtain a thin slice of the unfixed tissue. The liver section was cut while it was submerged in Anderson's solution; therefore, the tissue was never allowed to become dry. The thin section of liver floated to the surface and was picked up with a camel's hair brush and immediately transferred to a glass microscopic slide. The slide had been previously prepared by adding two drops of Anderson's solution. Pieces of a broken cover slip were placed at the edges of the drop and a cover slip was added; thus, the liver section was not under pressure because of the weight of the cover slip. One hundred living liver nuclei were measured with a filar micrometer eyepiece (#8105, Welch Scientific Company, Chicago) in order to establish a base or control to which fixed and stained nuclei could be compared. The liver nuclei were measured at random by using only those nuclei that were seen on the micrometer eyepiece scale. The microscope used was equipped with a mechanical stage and the microscopic slide was moved downward until the edge of the tissue was reached. The entire slide was then moved to the left, and the nuclei in this area were counted: thus, no nucleus was counted more than once.

The microscope used was a compound monocular microscope (Kassel Microscopes, Germany). The microscope was

equipped with a 10X eyepiece and a 100X oil immersion objective. The immersion oil was purchased from Cargille's Labs, Inc., New York. An incandescent light, 7½ watts, was used to illuminate the section.

For each nuclear measurement, maximum and minimum diameters were taken with the filar micrometer eyepiece; these two values were averaged and the volume determined as for a sphere. This step is justifiable according to Birge and Tibbitts.[1] The numbers obtained from the filar micrometer were converted to microns and the volumes were computed for each nuclei and expressed in cubic microns. The diameters and volumes obtained at this point were used as the control measurements for the unfixed nuclei.

There were five stages in the preparation of the tissues for study of microscopic sections. Each stage was compared to the control nuclei.

The first procedure in preparing the tissue was fixation. A piece of liver tissue, approximately 5 mm. thick, 6 mm. broad, and 10 mm. long, was transferred from Anderson's solution and was placed in 100 ml. of Bouin's picro-formol fixative[2] (General Biological Supply House, Chicago). The fixation process was maintained for 18 hours

---

[1]Birge and Tibbitts, op. cit., p. 409.

[2]Gray, op. cit., p. 74.

at room temperature.[1] A freehand section was cut, as previously described, from the liver tissue. A slide was perfused with Bouin's fixative and the section placed in it. The section was covered with a cover slip. A total of 100 fixed nuclei were measured as described above.

Washing was carried out in 70% ethyl alcohol. The washing process was prolonged until every visible trace of yellow due to the picric acid was washed out.[2] It was necessary to change the 70% ethyl alcohol at intervals due to the yellow coloration of the picric acid. Dehydration and hardening occurred as the liver tissue was passed through the ascending series of ethyl alcohols, each concentration increased by 5%, up to absolute ethyl alcohol (Chemline, American Drug Industries, Inc., Chicago). The tissue was left in each concentration of ethyl alcohol for 8 hours.[3] The volume of the mixture of the ethyl alcohol and water used at each concentration was 100 ml. The tissue was transferred to a second volume of absolute ethyl alcohol, which was necessary in order to remove all traces of

---

[1]A. B. Lee, The Microtomist's Vade-Mecum (Philadelphia: Blakiston Company, 1937), p. 38.

[2]A. E. Galigher, The Essentials of Practical Microtechnique in Animal Biology (Berkeley, California: Albert E. Galigher, Inc., 1934), p. 79.

[3]Ibid., p. 120.

water from the liver tissue. Three drops of absolute ethyl alcohol were placed on a slide and a freehand section was placed in it. While measuring the nuclei, it was necessary to add drops of ethyl alcohol to the edge of the cover slip because of the volatility of the reagent. A total of 100 washed and dehydrated nuclei were measured and their volumes were computed.

After all traces of water had been removed from the liver tissue, it was in a condition to absorb a liquid in which paraffin may subsequently be dissolved. Xylol (Mallinckrodt Chemical Works, St. Louis) was the solution used for clearing the liver tissue. Three graded mixtures of ethyl alcohol and xylol were used. The first mixture contained 3 parts absolute ethyl alcohol and 1 part xylol; the second, 2 parts absolute ethyl alcohol and 2 parts xylol; and the third, 1 part absolute ethyl alcohol and 3 parts xylol. The tissue was then placed in pure xylol. The volume used at each of the graded mixtures was 100 ml. A second volume of 100 ml. pure xylol must be used to insure the complete removal of ethyl alcohol. The tissue was left in each mixture for 8 hours.[1] If the tissues show whitish or opaque spots, the clearing was not complete. A slide was perfused with xylol and a section of the cleared liver

---

[1] Ibid., p. 122.

tissue was placed in the solution. The maximum and minimum diameters of 100 cleared hepatic nuclei were measured, and the nuclear volumes were calculated.

The transfer of the tissue from the clearing agent to paraffin was made gradually.[1] Soft paraffin (Matheson Chemical Company, Inc., Norwood, Ohio) which had a melting point range of 50-52° C. was used. This histowax was suitable for embedding tissues which must be cut into rather thick sections (14 microns or over). Finely shaved paraffin was added to the xylol. After all the paraffin was dissolved, more was added; the process was repeated until no more paraffin shavings dissolved. The liver tissue was left in this slushy condition for 18 hours. The cover of the container was removed so that the xylol could evaporate. The container was placed in an oven in which the temperature was maintained at 54° C.[2] After the slushy mixture melted, one half of the melted mixture was poured off and replaced with pure melted paraffin. The vial was gently agitated in order to thoroughly mix the two ingredients. The paraffin was changed twice; each time it was replaced with pure melted paraffin in order to remove all traces of the clearing agent. The tissue was left in the pure melted paraffin for 5 hours.

---

[1] Ibid., p. 124. [2] Ibid.

The inside of the embedding dish was smeared with glycerine. Pure melted paraffin was poured into the dish and the liver tissue was quickly transferred, using a warm forceps, to the prepared dish. The dish was moved to a pan containing cold water and the dish was immersed almost to its top. Cold water from the tap was allowed to flow into the pan; the surface was blown upon to hasten the cooling. A thick film was formed on the surface of the paraffin, the embedding dish was submerged, and the paraffin block floated to the surface.[1]

The sections were cut with a rotary microtome[2] (#820, General Biological Supply Company, Chicago). The thickness of each section was 15 microns. The sections were affixed to the slide using egg albumin[3] and were spread by passing the slides through the flame of a Bunsen burner several times. The slides, with the affixed sections, were placed in an oven at 37° C. for 20 minutes. The maximum and minimum diameters of 100 nuclei were measured and their volumes were computed.

The paraffin was removed from the section by passing the slide through pure xylol. The slide remained in the xylol for 3 minutes. The section was passed through a

---

[1] Ibid., p. 126.

[2] Guyer, op. cit., p. 37.

[3] Ibid., p. 40.

mixture of xylol and absolute ethyl alcohol (3 parts xylol, 1 part absolute ethyl alcohol), a second mixture of xylol and absolute ethyl alcohol (2 parts xylol, 2 parts absolute ethyl alcohol), and a third mixture (1 part xylol, 3 parts absolute ethyl alcohol), and finally into the absolute ethyl alcohol. In each of the preceding stages, the tissue remained in each solution for 3 minutes. Once the paraffin was removed, the section was rapidly transferred between solutions; thus, the section was never allowed to become dry.

The section was passed from the absolute ethyl alcohol to distilled water by using a descending series of ethyl alcohols.[1] The slide was left in each concentration for 3 minutes. The section was washed in running tap water for 5 minutes and then rinsed in distilled water.

The slide was transferred to freshly filtered 2% iron alum (Mallinckrodt Chemical Works) for 45 minutes.[2] The sections were rinsed in distilled water. The liver section was stained with 0.5% hematoxylin[3] (Harleco Parstains, Hartman-Leddon Company, Philadelphia). The tissue was destained in 2% iron alum until the section appeared gray. The section was rinsed in distilled water and washed in

---

[1]Galigher, op. cit., p. 132.

[2]Guyer, op. cit., p. 9.

[3]Ibid.

running water. The slide was perfused with distilled water and the diameters of 100 stained nuclei were measured and their volumes were calculated.

In this investigation, an adult male and an adult female mouse were used in order to ascertain differences in the tissue of the two sexes. Another purpose of the investigation was to determine if the same events would occur in the kidney tissue. The kidneys of the male and female mice were removed and passed through the same procedures as were the livers of the mice.

The kidneys were cut transversely and placed in Anderson's solution. A thin section was cut from the kidney and the nuclear diameter was measured. Only one measurement was taken for the renal nuclei since they were spherical. The nuclear diameter was measured in microns using the filar micrometer eyepiece and oil immersion objective of the microscope that was described previously. The volumes of these control nuclei were computed, using the formula for the volume of a sphere, and expressed in cubic microns.

The renal nuclei were fixed in Bouin's picro-formol solution. The time the tissues remained in the solutions in all of the following procedures was the same as that used with the liver tissue. In all cases, 100 renal nuclei were measured after each procedure in preparing the tissue and

the volumes were computed. The stages that followed the fixation were washing and dehydration in the ascending series of ethyl alcohols, clearing with xylol, embedding in paraffin, and staining with hematoxylin.

The procedure described should be adequate for one to compare the changes in diameters and volumes of the nuclei of the liver and kidney tissue. After each stage in preparing the tissues, an analysis of the data should show the amount of shrinkage or swelling that is evident. Opposite sexes of mice should indicate the changes in the nuclei of the liver and kidney tissues of each sex. Thus, the procedure used in this investigation should give an indication of the influence of certain chemicals in a standard procedure of the preparation of tissues for microscopic study.

CHAPTER IV

RESULTS AND INTERPRETATION OF DATA

As previously noted, the principal objective of the investigation presented herein was to ascertain the relative effect of various microtechnique methods used in the preparation of tissues for microscopic study. The amount of shrinkage and swelling at the different stages of fixation was measured and compared to the unfixed, control nuclei.

To determine the accuracy and the validity of the measurements, the diameters of 100 fixed liver nuclei were determined. The average diameter was computed to be 6.19 microns. The process was repeated and the average diameter of the nuclei was computed as 6.17 microns. This showed an error of 0.3% in reading the diameters of 200 nuclei.

During the measurement of the 100 liver nuclei in each of the stages in the microtechnique method, the ratio of diploid to tetraploid nuclei was in a range of 19-24% Class I or diploid nuclei to 76-81% Class II or tetraploid nuclei. Birge and Tibbitts, in their studies with liver tissue, found the percentage of diploid nuclei to be 27% and tetraploid nuclei to be 73%.[1] Anderson and Wilbur, in their studies of liver tissue, found the percentage of diploid

---

[1]Birge and Tibbitts, op. cit., p. 411.

nuclei to be 14% and tetraploid to be 86%. The unfixed, control nuclei of the liver tissue showed diploid and tetraploid percentages similar to those given for the unfixed nuclei.[1] Thus, the ranges of the two classes are consistent and would not result in a large error in nuclear diameter and volume measurements.

The unfixed hepatic nuclei were in the shape of an ellipse. A definite boundary, the nuclear membrane, was seen surrounding the nucleus. The nucleoplasm had a granular appearance.

As shown by Table I, in each stage of microtechnique tissue preparation, there was a definite amount of shrinkage. The over-all shrinkage in the nuclear diameter of hepatic cells from an adult male mouse was 29.9%. The greatest linear shrinkage occurred during fixation with Bouin's fluid and embedding with paraffin; this amounted to a decrease of 7.3% from the control and 13.7% from the cleared nuclei. In the remainder of the microtechnique process, the shrinkage was gradual and not excessive. The measurements for the nuclear diameter shrinkage of hepatic nuclei of the adult male mouse are summarized in Figure 1, page 38.

An over-all shrinkage of 29.9% in the diameter of the

---

[1] Anderson and Wilbur, op. cit., p. 790.

TABLE I

MEAN MEASUREMENTS OF THE NUCLEAR DIAMETERS AND THE PER CENT OF SHRINKAGE WHICH OCCURS IN THE VARIOUS STAGES OF MICRO-TECHNIQUE FOR LIVER AND KIDNEY TISSUES OF WHITE MICE AS COMPARED TO THE CONTROL NUCLEI

Source of nuclei	Diameter of control nucleus in microns	Bouin's		Alcohols		Xylol		Paraffin		Stain*	
		Diameter in microns	Shrinkage %	Diameter in microns	Shrinkage %	Diameter in microns	Shrinkage %	Diameter in microns	Shrinkage %	Diameter in microns	Shrinkage %
Liver Male	9.24	8.57	7.3	8.12	12.1	7.82	15.3	6.75	26.8	6.54	29.9
Liver Female	10.40	9.50	8.6	9.10	12.5	8.95	13.9	8.08	22.3	7.88	24.2
Kidney Male	10.36	8.43	18.6	7.57	26.9	7.38	28.8	6.13	40.8	5.96	43.4
Kidney Female	10.51	8.87	15.6	8.15	22.4	7.63	27.4	6.88	34.5	6.68	36.4

*Iron hematoxylin

liver nuclei of the male adult mouse corresponded to a decrease in volume of 64.5%. Table II shows the percentage of volume shrinkages for the nuclei of the liver and kidney tissues of the male and female mice. The measurements for the nuclear volume shrinkage of the hepatic nuclei of the male mouse are summarized in Figure 2, page 38.

As stated previously, the greatest shrinkage in the diameter of the hepatic nuclei from the male mouse occurred in the fixation and embedding stages. The same observation was made with the hepatic nuclei of a female adult mouse. Although, in the latter instance, it was evident that there was a greater shrinkage of the nuclei after fixation than after the embedding process. In the female, the shrinkage in diameter of the liver nuclei was 8.6% as compared with 7.3% in the liver nuclei of the male mouse. In the embedding stage, the greater shrinkage was observed in the diameter of the nuclei of the male, 26.8% as compared to 22.3% for the female liver nuclei. Patten and Philpott noted that the greater the shrinkage caused by fixation, the less the subsequent shrinkage.[1] In the various stages of preparation of the female liver tissue, the shrinkage was less than that of the preparation of the male liver tissue. Table I, page 35, shows percentage shrinkages of nuclear diameters

---

[1]Patten and Philpott, op. cit., p. 412.

TABLE II

MEAN MEASUREMENTS OF THE NUCLEAR VOLUMES AND THE PER CENT OF SHRINKAGE WHICH OCCURS IN THE VARIOUS STAGES OF MICRO-TECHNIQUE FOR LIVER AND KIDNEY TISSUES OF WHITE MICE AS COMPARED TO THE CONTROL NUCLEI

Source of nuclei	Volume of control nucleus in cubic microns	Bouin's		Alcohols		Xylol		Paraffin		Stain*	
		Volume in cubic microns	Shrinkage %	Volume in cubic microns	Shrinkage %	Volume in cubic microns	Shrinkage %	Volume in cubic microns	Shrinkage %	Volume in cubic microns	Shrinkage %
Liver Male	1,238.3	984.8	20.5	840.4	32.1	750.8	37.8	484.8	60.9	439.1	64.5
Liver Female	1,765.9	1,345.9	23.8	1,183.2	35.6	1,121.7	36.5	828.1	53.1	768.2	56.5
Kidney Male	1,744.9	937.5	46.3	678.5	61.1	631.3	63.8	359.7	79.4	332.3	81.0
Kidney Female	1,817.3	1,091.7	39.9	864.5	53.4	694.8	61.8	514.9	71.7	468.1	74.2

*Iron hematoxylin

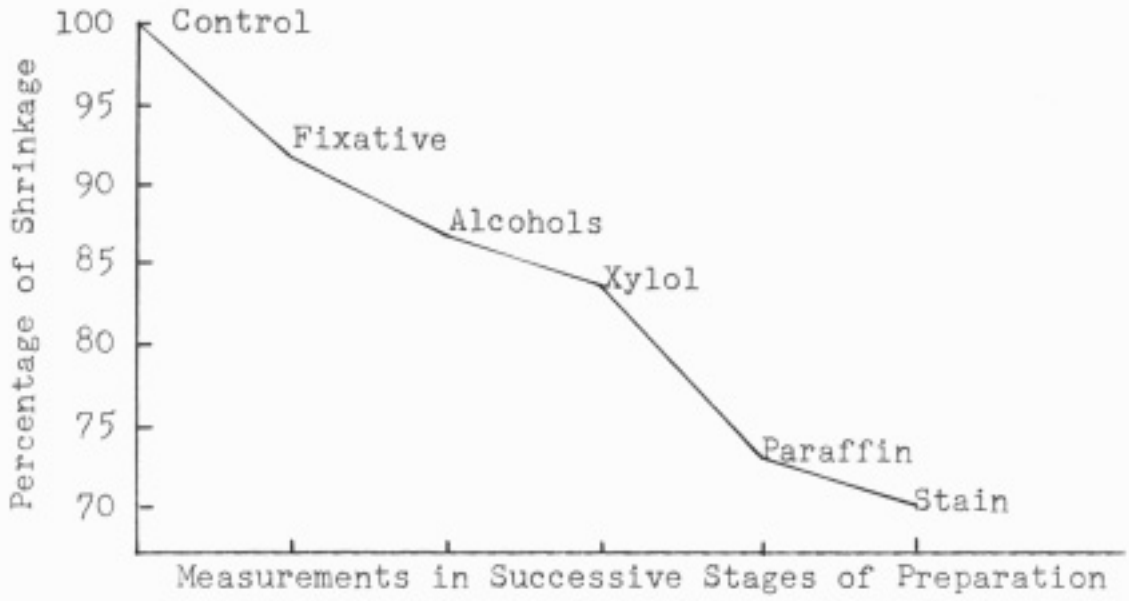

Figure 1. Average percentage of shrinkage induced in diameters of the hepatic nuclei of an adult male mouse by the various procedures used in microtechnique as compared to the control nuclei.

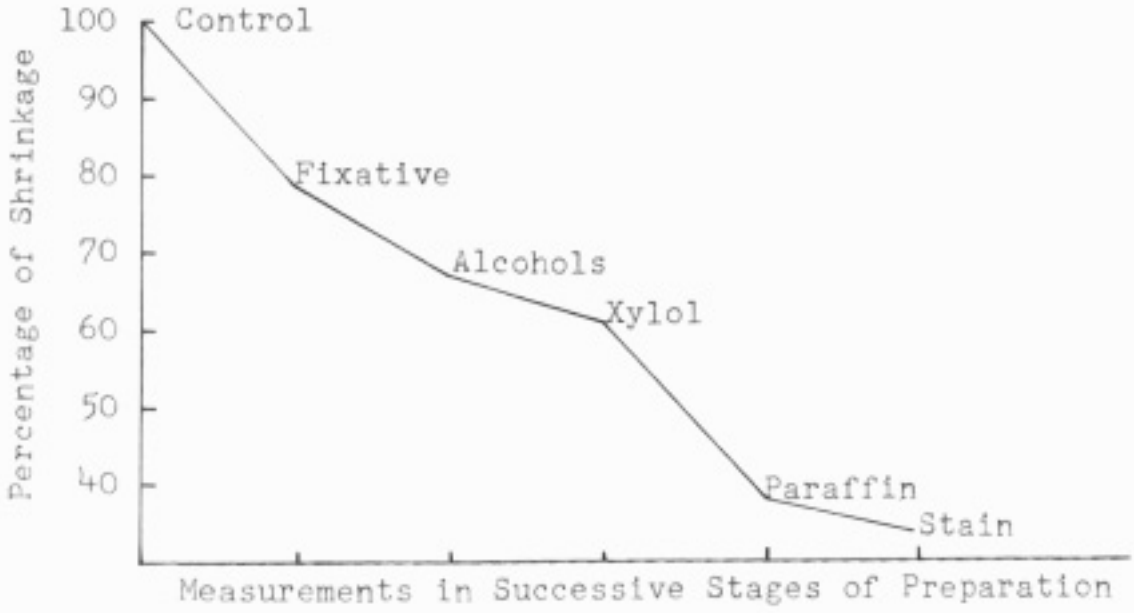

Figure 2. Average percentage of shrinkage induced in volumes of the hepatic nuclei of an adult male mouse by the various procedures used in microtechnique as compared to the control nuclei.

for each mouse and type of tissue.

It can be noted from Table I, page 35, that the hepatic and renal nuclei of the male mouse have a smaller diameter than that of the female mouse. The nuclear size is dependent to some extent on the sex and strain of the mouse used. Two mice were used in this experiment, a male weighing 26.3 g. and a female weighing 29.5 g. Thus, the difference in size of the mice may account for the difference in size of the nuclear diameters.

The hepatic nuclei of the female mouse followed approximately the same pattern of shrinkage as the hepatic nuclei of the male mouse. The greatest shrinkage occurred in the fixation and embedding stages. During fixation, the nuclear diameter decreased 8.6% and the nuclear volume decreased 23.8% in the hepatic cells. After the liver tissue had been embedded, the nuclear diameter had shrunk to 22.3% and the volume had shrunk to 53.1% of the original size. Over the entire range of microtechnique methods used for preparing the tissues, the nuclei of the female showed a smaller amount of shrinkage than the hepatic nuclei of the male. Figures 3 and 4 summarize the per cent shrinkage in nuclear diameter and volume.

The nuclei of the renal tissue showed a greater amount of shrinkage than those of the hepatic tissue. Examination of Table III, page 41, will indicate the total

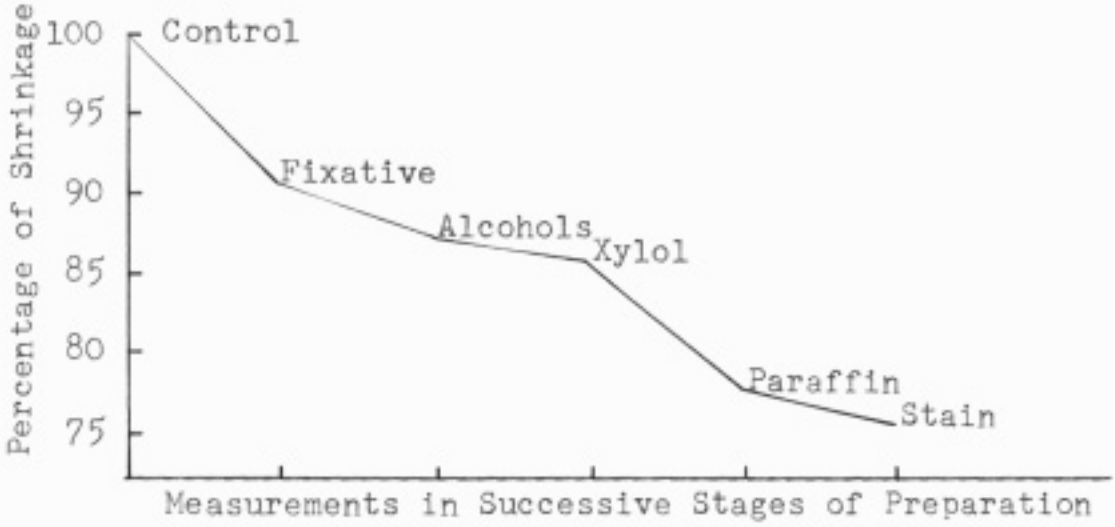

Figure 3. Average percentage of shrinkage induced in diameters of the hepatic nuclei of an adult female mouse by the various procedures used in microtechnique as compared to the control nuclei.

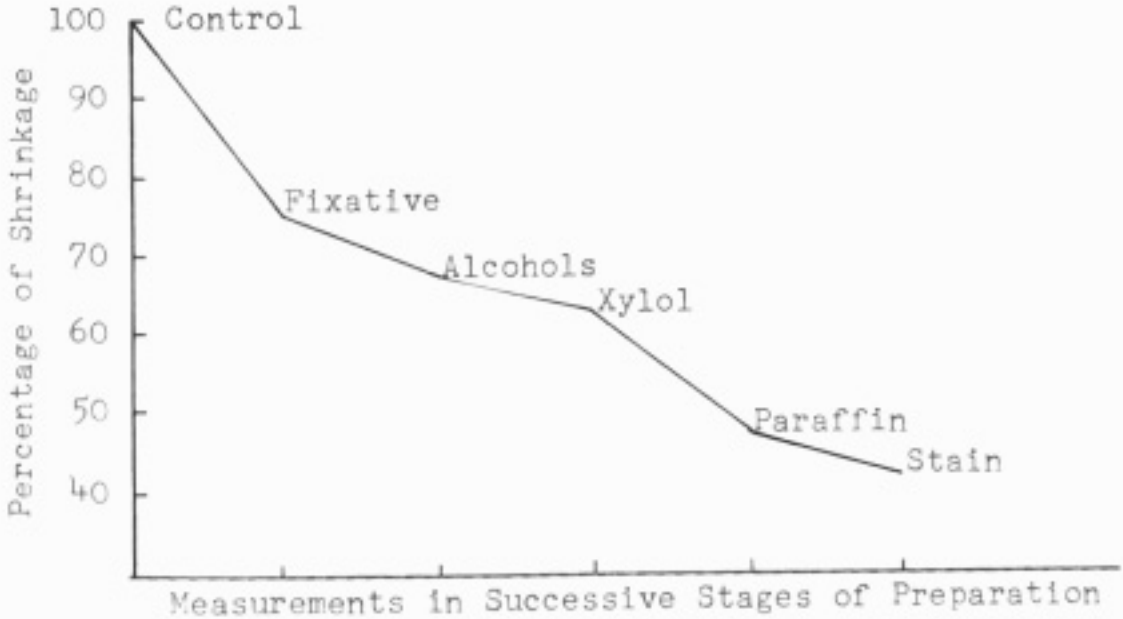

Figure 4. Average percentage of shrinkage induced in volumes of the hepatic nuclei of an adult female mouse by the various procedures used in microtechnique as compared to the control nuclei.

TABLE III

TOTAL SHRINKAGE OF THE NUCLEI FOR THE KIDNEY AND LIVER TISSUES OF WHITE MICE AFTER ALL STAGES OF THE MICROTECHNIQUE METHOD

Tissue	Per cent. Shrinkage in diameter	Per cent. Shrinkage in volume
Liver, Male Adult Mouse	29.9	64.5
Liver, Female Adult Mouse	24.2	56.5
Kidney, Male Adult Mouse	43.4	81.0
Kidney, Female Adult Mouse	36.4	74.2

linear and volume shrinkages. Also, it can be seen that the renal nuclei of the female mouse shows less shrinkage than the renal nuclei of the male mouse. The total volume decrease in the renal nuclei of the male mouse was 81.0%, whereas, in the renal nuclei of the female, the decrease was 74.2%. The above observation is also shown in the liver nuclei; the volume decrease in the liver nuclei of the male mouse was 64.5% and in the female 56.5%.

The greatest shrinkage of any of the nuclei occurred in the kidney nuclei of the adult male mouse when it was placed in Bouin's fixative. The nuclear diameter decreased 18.6% and the nuclear volume decreased 46.3%. This is illustrated by the graphs of Figures 5 and 6.

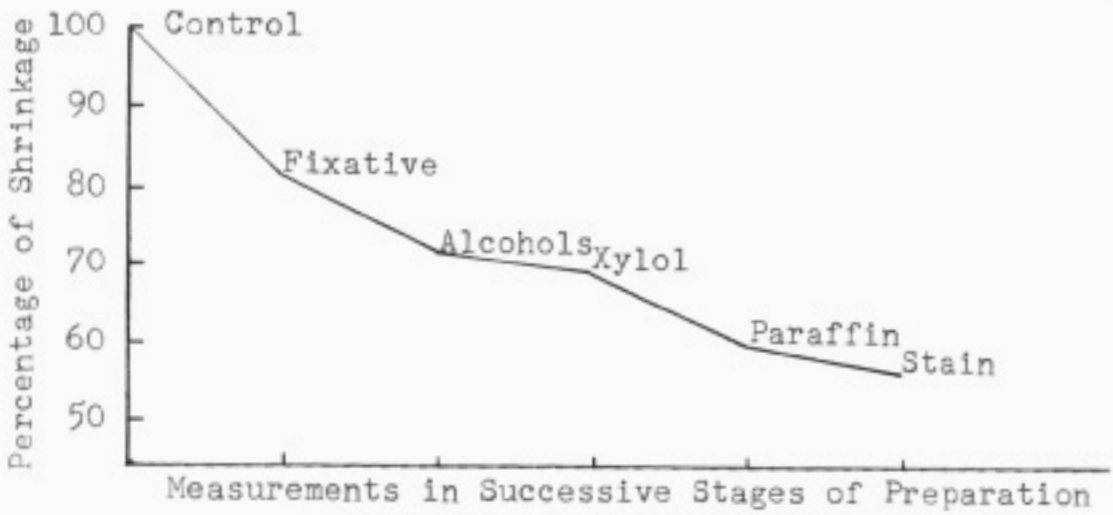

Figure 5. Average percentage of shrinkage induced in diameters of the renal nuclei of an adult male mouse by the various procedures used in microtechnique as compared to the control nuclei.

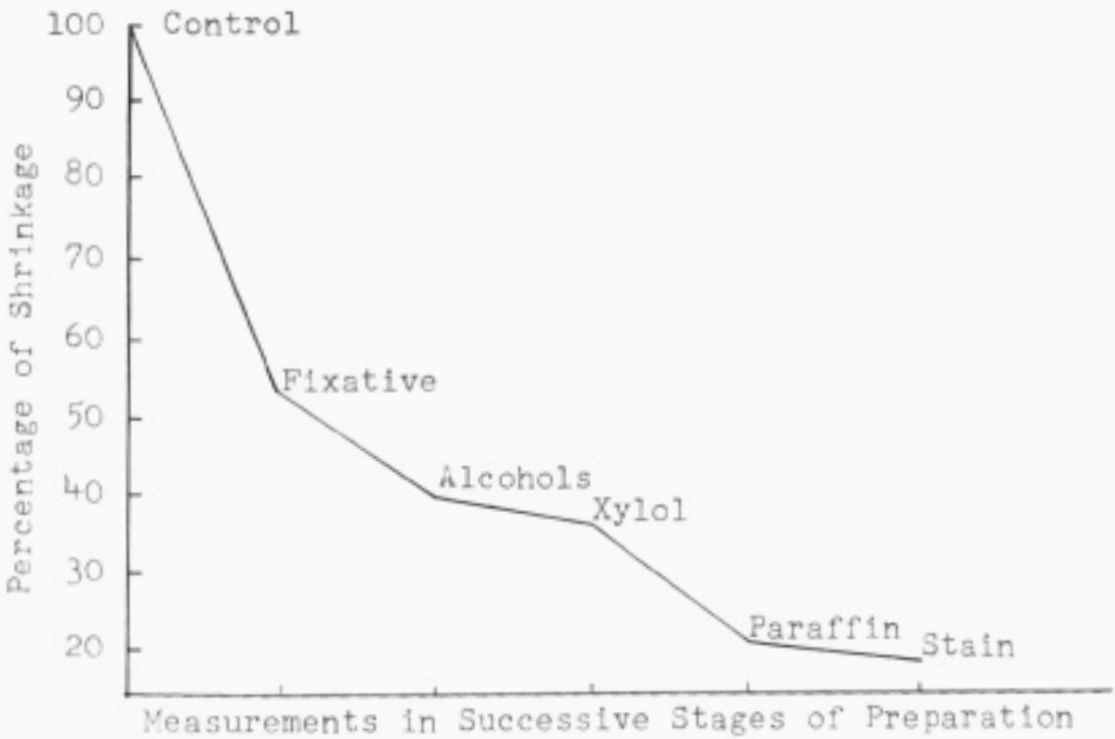

Figure 6. Average percentage of shrinkage induced in volumes of the renal nuclei of an adult male mouse by the various procedures used in microtechnique as compared to the control nuclei.

A large amount of shrinkage also occurred when the kidney nuclei of the female mouse were subjected to the influence of fixation. The nuclear diameter decreased 15.6% and the nuclear volume decreased 39.9%. This is shown by the graphs of Figures 7 and 8.

Ross and Birge and Tibbitts noted that fixation was probably responsible for most of the shrinkage in a tissue preparation.[1] The results of this investigation are in agreement with their conclusions. A large portion of the shrinkage was observed in this step for all the mice regardless of the kind of tissue studied. Patten and Philpott measured pig embryos after fixation with Bouin's. They observed a very slight shrinkage (2.5%).[2] This was a very small shrinkage compared with the results of this investigation. However, the penetration of the fixative would not be sudden and complete in the entire pig embryo as it was in the small pieces of the excised liver and kidney used in this investigation.

The effect of dehydration using the ascending series of ethyl alcohols did not decrease the diameters and volumes of the liver nuclei excessively. As shown in Tables I and II, pages 35 and 37, the nuclear diameter decreased 4.9% in

---

[1]Ross, op. cit., p. 125; and Birge and Tibbitts, op. cit., p. 409.

[2]Patten and Philpott, op. cit., p. 407.

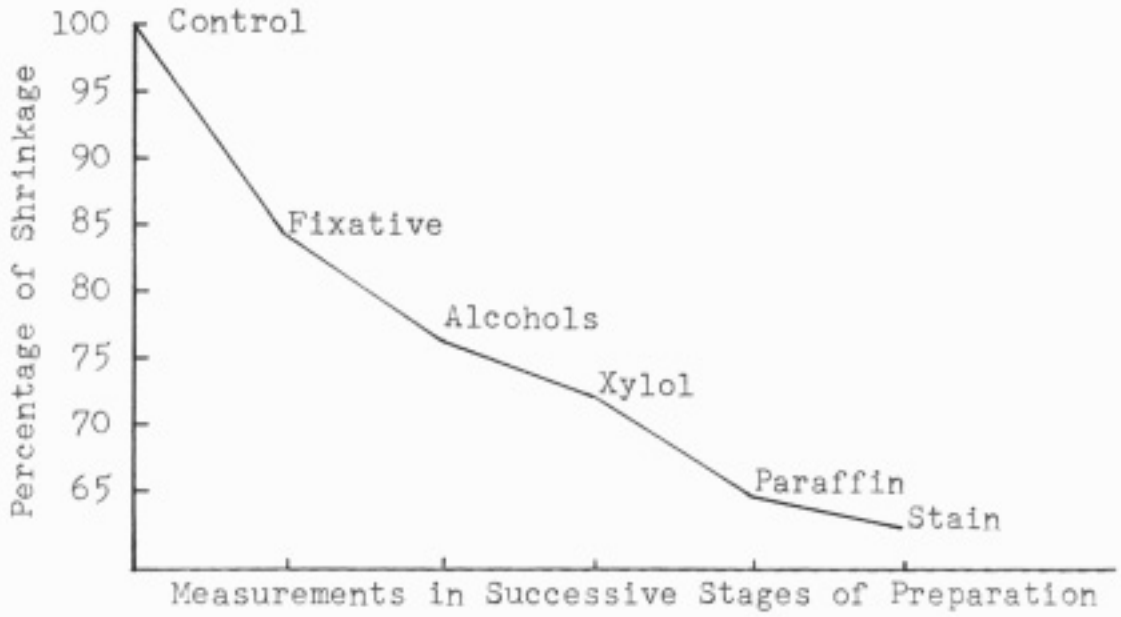

Figure 7. Average percentage of shrinkage induced in diameters of renal nuclei of an adult female mouse by the various procedures used in microtechnique as compared to the control nuclei.

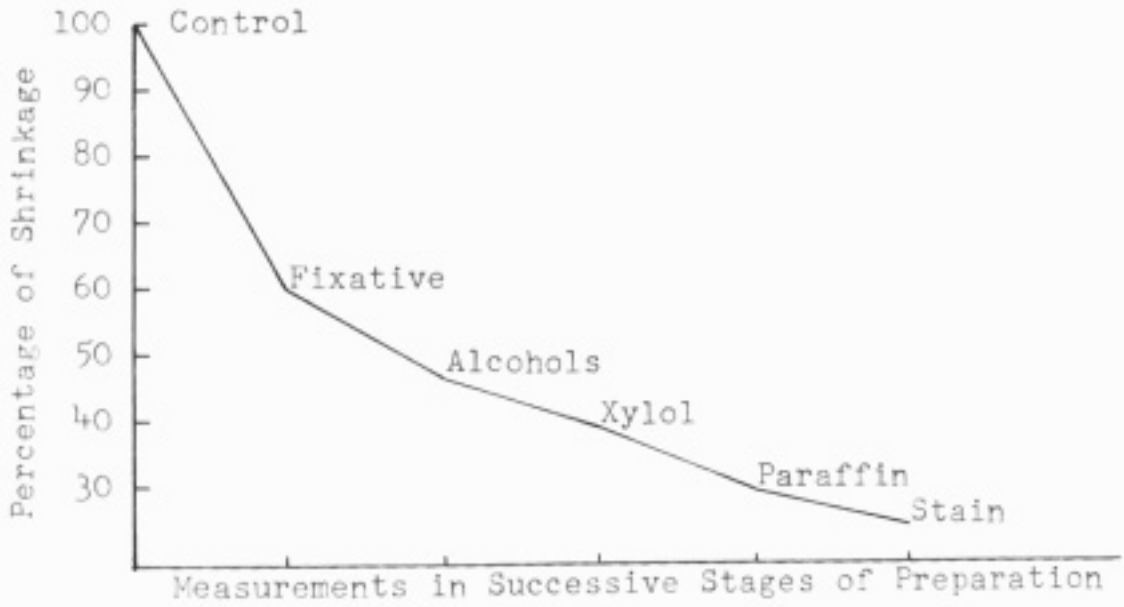

Figure 8. Average percentage of shrinkage induced in volumes of renal nuclei of an adult female mouse by the various procedures used in microtechnique as compared to the control nuclei.

the male mouse and 3.9% in the female mouse; the nuclear volume decreased 11.7% in the male and 9.8% in the female. The effect of dehydration was more marked in the kidney tissue. The nuclear diameter decreased 8.3% in the male and 6.8% in the female and the nuclear volume decreased 14.8% in the male and 13.5% in the female during the dehydration process. The shrinkage due to the alcohols is summarized by the graphs of Figures 1 through 8.

Tarkhan noted that alcohol was perhaps the most powerful simple fixative.[1] In this investigation, alcohol was used as the dehydrating agent and the per cent of shrinkage of liver and kidney tissues was not great. It may be postulated that, since much of the shrinkage occurs in the fixation process, the effect of alcohol will not be great.

The process of clearing tissues showed a slight shrinkage. The effect of xylol on the tissues is indicated by the graphs of Figures 1 through 8. The least amount of shrinkage due to xylol was 1.5% decrease in the nuclear diameter of the liver tissue of the female mouse. The greatest percentage change due to xylol occurred in the kidney nuclei of the female mouse; this amounted to a decrease in the nuclear diameter of 5.0%. Patten and

---

[1]Tarkhan, op. cit., p. 391.

Philpott observed a shrinkage of only 1% or less in the pig embryos when using xylol as the clearing agent following Bouin's fixative.[1] In this investigation, the least amount of shrinkage in any of the stages in the microtechnique method was noted in the clearing process.

Tarkhan and Ross postulated that the embedding process was one of the most important factors in determining the final cell size.[2] In this investigation, the greatest shrinkage of the tissues occurred upon embedding the tissue in paraffin. As noted in the liver nuclei, the greatest decrease in nuclear diameters and volumes was observed in the fixation process and the evidence for embedding paralleled these results. This observation could also be shown for the renal nuclei.

Table I, page 35, records the per cent shrinkage of nuclear diameters for the embedding process. The liver nuclei showed a shrinkage of 11.5% in the male and 8.4% in the female. The shrinkage in the diameter for the kidney nuclei was computed to be 12.1% in the male mouse and 7.1% in the female. Figures 1 through 8 show the decrease in diameter and volume during the embedding process.

The process of staining did not produce an appre-

---

[1]Patten and Philpott, op. cit., p. 406.

[2]Tarkhan, op. cit., p. 396; and Ross, op. cit., p. 138.

ciable effect on the shrinking of the nuclei. Cowdry determined that there was a shrinkage of more than 10% in the staining process.[1] The greatest shrinkage observed was 3.1% in the liver nuclei of the male mouse. Cowdry determined the shrinkage by placing the stain directly on the excised tissue; thus, no initial shrinkage had taken place.[2]

All nuclei measured in this investigation showed a definite margin. Irregular or distorted nuclei were not included in the data. The nuclei counted were obtained by a random sampling. No attempt was made to study specific nuclei of the liver, such as parenchymal or non-parenchymal, but samples were taken from all parts of the section. The nuclei studied from the kidney section were from the cortical region.

---

[1] Cowdry, loc. cit.

[2] Ibid.

CHAPTER V

CONCLUSIONS

The principal objective of this study was to determine the relative effect of the various processes that produced shrinkage or swelling of liver and kidney nuclei during fixation or in the subsequent processing of tissues by the paraffin method.

Samples of mice liver and kidney were fixed in Bouin's-Picro-Formol solution, dehydrated and hardened in an ascending series of alcohols, cleared with xylol, embedded in paraffin, and stained with iron hematoxylin. By a comparison of liver and kidney tissues, it was noted that the fixative and paraffin embedding stages caused the greatest shrinkage in both types of tissues. The most shrinkage occurred in the Bouin's fixative.

It was also shown by this investigation that the shrinkage during dehydration, clearing, and staining was not excessive. When the shrinkage was less in Bouin's reagent, the decrease in nuclear size was greater in the following stages. This may be attributed to the fact that the limit to which tissue may shrink had not been exhausted; thus, the subsequent processing would shrink the nucleus to this limit.

The average shrinkage of diameter and volume was

computed in per cent and, from these averages, graphs were prepared to show the shrinkage encountered at each step of the technique used.

The size of the liver and kidney nuclei of the female mouse was larger than that of the male. The total decrease in diameter and volume of the kidney nuclei was greater than the total decrease of the liver nuclei.

All the reagents used in paraffin-wax embedding, ethyl alcohol, xylol, molten paraffin-wax, and the stain tend to shrink cells. The over-all effect of these reagents, together with the fixative, is to reduce the diameter of the nucleus about one-third of what it was in a living state.

Small pieces of tissue were used in this investigation, and the reagents, for all practical intents and purposes, had almost immediate access to every cell. It is uncertain whether the same results would be obtained in the case of cells deeply embedded in relatively large pieces of compact organs, such as the entire kidney or liver.

Further investigation might be carried out to evaluate the greater shrinkage of the nuclei from tissues of the female mouse as compared to a smaller shrinkage in diameter of the tissue from the male mouse.

BIBLIOGRAPHY

BIBLIOGRAPHY

A. BOOKS

Conn, H. J. Biological Stains. Geneva, New York: Biotech Publications, 1946.

Cowdry, E. V. Microscopic Techniques in Biology and Medicine. Baltimore: The Williams and Wilkins Company, 1943.

Galigher, A. E. The Essentials of Practical Microtechnique in Animal Biology. Berkeley, California: Albert E. Galigher, Inc., 1934.

Gray, Peter. Handbook of Basic Microtechnique. New York: McGraw-Hill Book Company, Inc., 1958.

Guyer, Michael F. Animal Micrology. Chicago: The University of Chicago Press, 1953.

Lee, A. B. The Microtomist's Vade-Mecum. Philadelphia: Blakiston Company, 1937.

McClung, C. E. Handbook of Microsconical Technique. New York: Paul B. Hoeber, Inc., 1937.

B. PERIODICALS

Anderson, N. G., and K. M. Wilbur. "Studies on Isolated Cell Components. IV. The effect of various solutions on the isolated rat liver nucleus," Journal of General Physiology, XXXV (May, 1952), 781-796.

Aumonier, F. J. "Notes on the Distortion of Paraffin Sections," Journal of Royal Microscopical Society, LVIII (1938), 253-257.

Birge, Wesley J., and F. Donald Tibbitts. "The Use of Sodium Chloride-Containing Fixatives in Minimizing Cellular Distortion in Histological and Cytochemical Preparations," Journal of Histochemistry and Cytochemistry, IX (April, 1961), 409-414.

Freeman, B. L., E. K. Moyer, and A. M. Lassek. "The pH of Fixing Fluids During Fixation of Tissues," Anatomical Record, CXXI (March, 1955), 593-600.

Koenig, H. R., A. Groat, and W. F. Windle. "A Physiological Approach to Perfusion Fixation of Tissues with Formalin," Stain Technology, XX (January, 1945), 13-22.

Lassek, A. M., and M. M. Powers. "Concentrated Formalin Versus a 10% Solution as a Fixative Preceding Silver Staining," Stain Technology, XXIV (January, 1949), 33-37.

Patten, B. M., and R. Philpott. "The Shrinkage of Embryos in the Processes Preparatory to Sectioning," Anatomical Record, XX (March, 1922), 393-413.

Ross, K. F. A. "Cell Shrinkage Caused by Fixatives and Paraffin-Wax Embedding in Ordinary Cytological Preparations," Quarterly Journal of Microscopical Science, XCIV (1953), 125-129.

Stearn, A. E., and E. W. Stearn. "The Mechanism of Staining Explained on a Chemical Basis. I. The reactions between dyes, proteins, and nucleic acids," Stain Technology, IV (October, 1929), 111-119.

Stowell, Robert E. "Effect on Tissue Volumes of Various Methods of Fixation, Dehydration, and Embedding," Stain Technology, XXVI (April, 1941), 67-84.

Tarkhan, A. A. "The Effect of Fixatives and Other Agents on Cell Size and Tissue Bulk," Journal of Royal Microscopical Society, LI (1931), 387-400.

Tolstoouhov, A. V. "The Effect of Preliminary Treatment (Fixing Fluids) on Staining Properties of Tissues," Stain Technology, III (April, 1928), 49-56.

Wilson, J. W., and E. H. Leduc. "Nuclear Phenomena in Mouse Liver," American Journal of Anatomy, LXXXII (May, 1948), 353-362.

Zollinger, H. U. "Cytological Studies with the Phase Microscope. Alterations in the Nuclei of 'Resting' and Dividing Cells Induced by Means of Fixatives," American Journal of Pathology, XXIV (October, 1948), 798-806.